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Legionellosis in Patients with HIV Infection

Summary: During the five-year period 1984–1988 we received 192 specimens from 180 patients infected with the human immunodeficiency virus (HIV) for investigation of Legionella infection. The majority of specimens were bronchoalveolar lavage (BAL) fluids (84%), but tracheal suction and lung tissue from autopsies were also examined. The diagnostic methods used were a direct immunofluorescence assay (DFA) for the detection of Legionella antigen, and culture on buffered charcoal yeast extract (BCYE-α) media. All specimens were also examined for the presence of other bacterial lung pathogens, and all BAL specimens additionally for Pneumocystis carinii and mycobacteria. Legionellosis was not found to be common among HIV-infected patients, as only six specimens (3%) from six patients were found positive by DFA, and no specimens were culture-positive for Legionella species. Dual infection with Legionella and P. carinii occurred in two patients. Clinical data of the six patients are presented, and currently used methods for diagnosing legionellosis are discussed.


Introduction

Patients infected with the human immunodeficiency viruses (HIV) are susceptible to a wide range of bacterial and non-bacterial complications in addition to their primary infection. Pneumonia caused by Pneumocystis carinii is the most common opportunistic infection in North American and European HIV patients [1, 2], but other microorganisms, such as mycobacteria [3] and cytomegalovirus, are also important lung pathogens in this group of patients. Since several members of the genus Legionella are well-known causes of life-threatening pneumonia in immunocompromised patients [4], we found it of interest to review our results from investigating respiratory tract specimens from patients with HIV for Legionella species during the last five years. Previously reported legionellosis in patients infected with HIV include a few case histories [5–8], and more extensive surveys of bacterial infections in patients with the acquired immunodeficiency syndrome (AIDS) [1, 9–12]. This is to our knowledge the largest European survey of legionellosis in HIV-infected patients.

Materials and Methods

During the period of January 1, 1984 to December 31, 1988 we received a total of 651 samples for culture and antigen detection from patients suspected of legionellosis.

All specimens were investigated by Direct Fluorescent Antibody (DFA) testing, until August 1, 1988, with polyclonal antibodies to Legionella pneumophila serogroups 1–4, L. pneumophila serogroups 5–6, Legionella micdadei, Legionella bozemanii, Legionella dumoffii, and Legionella gormanii (Organon Teknika, Charleston, South Carolina, USA). In case of a positive reaction with one of the pooled L. pneumophila conjugates the specimen was retested with conjugates representing the individual serogroups. From August 1, 1988 we changed to using one single reagent, a monoclonal antibody directed against all serogroups of L. pneumophila (Genetic Systems, Seattle, Washington, USA). The corresponding polyclonal and monoclonal antibodies are considered to have equal sensitivity, the monoclonal antibody being the more specific without any of the (rarely occurring) cross-reactions described for the polyclonal L. pneumophila antibodies (Pseudomonas spp., Bacteroides fragilis, Flavobacterium spp.) [13]. Criteria of positivity was the presence of more than five brightly fluorescing (3+ or 4+) Legionella-like organisms per field.

All specimens were cultured on buffered charcoal yeast extract media containing alpha-ketoglutarate (BCYE-α). Blood agar was used as a control. From August 1, 1988 we included culture on modified Wadowsky-Yee (MXY) media containing polymyxin B, vancomycin and cycloheximide. Plates were incubated at 35°C in a humid atmosphere and inspected daily for seven days.

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Table 1: Microbiological findings, treatment and outcome in HIV-infected patients with a positive DFA for *Legionella* species.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>Specimen positive in DFA</th>
<th><em>Legionella</em> species</th>
<th>Other pathogens present</th>
<th>Antibiotic treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>Lung tissue (postmortem)</td>
<td><em>Legionella pneumophila</em> + <em>Legionella micdadei</em></td>
<td>None</td>
<td>Nonea</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>Lung tissue (postmortem)</td>
<td><em>Legionella micdadei</em></td>
<td><em>Pneumocystis carinii</em></td>
<td>Pentamidinea</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>BALb</td>
<td><em>Legionella pneumophila</em></td>
<td><em>Haemophilus influenzae</em>  <em>Staphylococcus aureus</em></td>
<td>Erythromycin  Fusidic acid  Flucloxacillin</td>
<td>Recovered</td>
</tr>
<tr>
<td>4</td>
<td>51</td>
<td>BAL</td>
<td><em>Legionella pneumophila</em></td>
<td><em>Pneumocystis carinii</em></td>
<td>Erythromycin  + TMP-SMZc</td>
<td>Died after 2 weeks of Kaposi sarcoma</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>BAL</td>
<td><em>Legionella pneumophila</em></td>
<td>None</td>
<td>None</td>
<td>Alive and well</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>BAL</td>
<td><em>Legionella pneumophila</em></td>
<td>None</td>
<td>Erythromycin</td>
<td>Died after 2 months of probable cerebral toxoplasmosis</td>
</tr>
</tbody>
</table>

a At the time of death;  
b BAL = bronchial lavage fluid;  
c TMP-SMZ = trimethoprim-sulfamethoxazole.

All specimens were also examined for the presence of other bacterial pathogens, and all bronchoalveolar lavage specimens and lung biopsies were additionally examined for *P. carinii* by silver methenamine stain, mycobacteria by Ziehl-Neelsen stain, and cryptosporidia by a modified Ziehl-Neelsen staining method. Culture for cytomegalovirus, herpesvirus, and mycobacteria was performed at Statens Seruminstitut, Enterovirus Department and Mycobacteria Department, respectively. Serum samples were investigated for anti-*Legionella* antibodies by a microagglutination (MA) test used routinely in our laboratory [14].

**Results**

We examined 192 specimens originating from 180 patients with fulminant AIDS or other manifestations of documented HIV infection. One hundred and sixty-two specimens (84%) were bronchoalveolar lavage (BAL) fluids, 17 (9%) of the specimens lung tissue, six (3%) tracheal suction samples, and four (2%) sputum samples. Six specimens (3%) from six patients were found positive for *Legionella* by DFA, two of which were lung biopsies obtained at autopsy; no *Legionella* species could be cultured, and determination of anti-*Legionella* antibodies by MA gave negative results for the five patients tested. In two of the five patients investigated, however, the serum samples available were taken before or just at the onset of disease, where a positive *Legionella* antibody titre could not be expected under any circumstances. In two specimens positive for *Legionella* the presence of *P. carinii* was also demonstrated. Mycobacteria, cytomegalovirus- or herpesvirus were not cultured from any of the specimens examined. After reviewing the charts of the six patients, a clinical diagnosis of pneumonia with fever, dyspnoea and cough at the time of the positive DFA could be established in five cases. The sixth patient was hospitalized because of thoracic pain and fever, but at the time of bronchoscopy he was afebrile and had a normal chest roentgenogram. Subsequently he was not treated with antibiotics (Table 1) and recovered uneventfully. The two patients in whom legionellae were detected post mortem had not received erythromycin therapy at the time of death, but one of them had been treated for a week prior to death with erythromycin on clinical suspicion of *Legionella* infection. The remaining three patients were treated with the recommended dosage of erythromycin (1 g x 4) with ensuing clinical effect, defined as regression of fever and pulmonary symptoms within three days. Two of the patients eventually died, one from progressive Kaposi sarcoma, the other possibly from cerebral toxoplasmosis complicated by bronchopneumonia.

**Case 1**

Thirty-eight-year-old homosexual man; anti-HIV antibodies found in March 1985 when the patient had suffered from fever, lymphadenopathy, weight loss and skin rash for about a year. The patient was on a continuous prophylactic trimethoprim-sulfamethoxazole (TMP-SMZ) dose after an episode of pneumonia of unknown etiology. Shortly after two weeks' hospitalization for an acute bleeding gastric ulcer, the patient was readmitted in poor condition with fever, dyspnoea, cough and thoracic pain. At this point the patient was still being treated with TMP-SMZ 160 mg/800 mg x 2. Since a systemic fungal infection was suspected, treatment with amphotericin B and flucytosine was begun. This had no effect on the fever and lung symptoms and thus the TMP-SMZ dose was increased to 320 mg/1,600 mg x 4. The patient's temperature curve showed a septic course, and on suspicion of bacteremia cefotaxime 2 g x 3 and netilmicin 100 mg x 3 was instituted. The patient's condition, however, deteriorated rapidly,